Modeling the Nanoenvironment of Biological Reactions to Enable Greater *in vivo* Relevance to *in vitro* Measurements

NIST scientists have developed a model system that mimics the "crowded" nanoenvironment in which protein enzymatic reactions take place in the cell. The new system enables NIST to perform isothermal calorimetry measurements of enzyme activities and obtain readings that more accurately reflect the characteristics of enzymes as they function in the cell. The ability to obtain more biologically relevant enzyme activity measurements is expected to enable new innovation in biotechnology and bioenergy.

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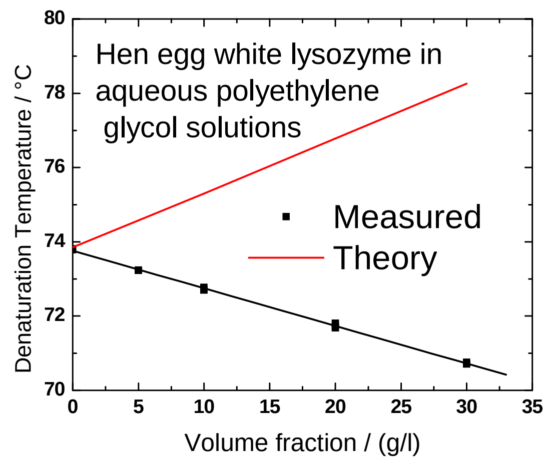

Thermodynamic measurements of biochemical reactions are typically conducted in very dilute solutions, but this does not reflect actual *in vivo* conditions where other, nonreacting molecules are present. There are *in vivo* system effects, which influence the thermodynamic properties of biochemical reactions, but which are not quantified at all in most *in vitro* biochemical studies of thermodynamics and kinetics. Providing a framework that allows prediction of the system effects on biochemical reactions is the goal of this project. We have developed new facilities in biological calorimetry to study such "macromolecular crowding phenomena." In FY '06 we set up a new laboratory and carried out initial measurements on the denaturation of lysozyme protein. Addressing these needs is well matched to NIST's expertise in both biochemical measurements and solution physics.

More physiologically relevant protein function measurements are now possible with NIST's nanomolecular crowding experimental system. More accurate enzyme thermodynamic measurements are being conducted.

NIST has begun a program to facilitate the use of existing thermodynamic data for prediction of *in vivo* processes. Thermodynamic measurements of biochemical reactions are conducted *in vitro*, in very dilute solutions. Actual biochemical reactions occurring in physiological milieu find themselves in the presence of a very high volume percentage of biomolecules that are non-specific to the reaction being studied, a situation very unlike the *in vitro* conditions of the thermodynamic measurements. Awareness is growing that these additional molecules affect quite significantly the biochemical reactions, theoretically by pushing them towards a state with smaller molecular sizes. In other words, a chemical reaction that reduces significantly the size of the product molecules, compared to the reactants, should be pushed further towards completion by the presence of a large volume fraction of non-reacting biomolecules than would be the same reaction in a very dilute solution, as in the *in vitro* measurement. Similarly, reactions that increase the molecular sizes of products over that of reactants should be inhibited by the presence of the additional, non-reacting biomolecules. These general effects are referred to as "macromolecular crowding."

Systematic studies are essential to improve and test theories of the effects of "crowding" on biochemical reactions and to facilitate the use of the large archive of biochemical data. The Physical and Chemical Properties Division has developed new facilities in biological calorimetry and has initiated experimental studies on macromolecular crowding phenomena. The Division has obtained a biological differential scanning calorimeter and an isothermal titration calorimeter, and has placed those instruments into operation. They are now being used to answer fundamental questions about macromolecular crowding. Calorimetry is an essential tool in separating entropic effects from specific energetic (enthalpic) effects. Calorimetry can usually provide more accurately the entropy and enthalpy of a reaction than is obtained by other means. As such, calorimetry can uncover smaller specific enthalpies of interaction – that invalidate the application of theory – than can other measures of biochemical reactions.

There are several open questions regarding the measurements of "crowding" in the first place. One of these is the concept of a perfect simulator molecule that can mimic physiological crowding. A molecule that simulates crowding should have no specific interaction with any of the components of the biochemical reaction that is being measured. Several different materials have been used to mimic physiological crowding. One of the most popular crowding simulators is polyethylene glycol, which has been used in more than half of the biochemical studies investigating the effects of crowding on individual biochemical reactions. We examined the effect of polyethylene glycol on the thermal denaturation of a protein, lysozyme. Denaturation of a protein occurs often with a significant change in the protein's gyration radius and therefore should be affected by macromolecular crowding. The temperature at which the protein denatured decreased with increasing concentration of polyethylene glycol, completely opposite to the prediction of theory (see figure). This result shows that polyethylene glycol is not free of interaction with at least some proteins and so it may be of questionable use as a crowding simulator. Studies of the effect of other crowding simulators on protein stability will be pursued in FY07.